The effects of refrigerated and frozen storage on Holder pasteurized donor human milk: A systematic review

By: Hannah R. Schlotterer and Maryanne T. Perrin

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Abstract:

Background: Pasteurized donor human milk (PDHM) is the recommended feeding alternative for preterm infants when mother's own milk is not available. Use of PDHM in United States neonatal hospitals is increasing, although guidelines for the refrigerated and frozen storage are limited. ***Objective:*** We aimed to review the current evidence for the storage of Holder PDHM (HPDHM) under refrigerated and frozen storage conditions. ***Methods:*** A systematic review of the literature was conducted for studies published between 1985 and May 2018. Studies were included if they studied the storage of Holder-pasteurized human milk under refrigerated or frozen storage conditions. ***Results:*** Fourteen studies met the inclusion criteria. Five studies addressed refrigerated storage and nine studies addressed frozen storage. There was little overlap in the outcomes measured or the analytical methods employed. There was concordance in three studies reporting no microbial growth over 4–9 days of refrigerated storage, and in five studies reporting a reduction in fat during 1–8 months of frozen storage. Only one study assessed the storage of HPDHM that had been fortified. ***Conclusions:*** Long-term refrigerated and frozen storage of HPDHM affects some components in milk more than others. While there is evidence of microbial purity during four or more days of refrigerated storage in clinical conditions, there is limited research on the impact of macro and micronutrients, or the impact of fortifiers. More research is needed in these areas.

Keywords: Holder-pasteurized donor human milk | donor milk | storage | refrigerated | frozen

Article:

Introduction

According to a 2017 policy statement published by the American Academy of Pediatrics, the use of pasteurized donor human milk (PDHM) distributed by milk banks is recommended for infants weighing <1,500 g when a mother's own milk is unavailable or insufficient.[1] The use of PDHM is associated with reduced healthcare costs,[2] better health outcomes,[3–5] and a reduced risk of the

development of necrotizing enterocolitis.[6] Results from the Center for Disease Control's 2015 Maternity Practices in Infant Nutrition and Care survey indicated that, two-thirds of neonatal intensive care hospitals were using donor human milk, and over 90% were using fortifiers to increase the nutrient content of human milk (HM).[7]

The pasteurization of donor HM is common practice among milk banking organizations, primarily to remove infectious contaminants and reduce the growth of harmful bacteria.[8,9] This is an appropriate concern given that PDHM is often fed to preterm infants who have compromised immune systems.[10] The Holder method of pasteurization is the predominating procedure used by milk banks internationally and involves heating the HM to 62.5–63°C for 30 minutes.[11–14] Holder pasteurization of HM has been well studied. While the process does indeed partially diminish several important biological components, such as immunoglobulins and the activity of lipase enzymes, macronutrients are almost fully retained.[9]

Less is known about what happens to Holder PDHM (HPDHM) over the course of long-term storage. While the Academy of Breastfeeding Medicine has issued storage recommendations for raw HM fed to healthy infants in home settings,[15] recommendations regarding the storage of HPDHM for use in a clinical setting with medically fragile infants are scarce. Understanding appropriate storage conditions for HPDHM in a clinical setting is an important area of research.

In 2011, the Human Milk Banking Association of North America (HMBANA) issued guidelines recommending that thawed HPDHM should be stored at 4°C and used within 24 hours.[11] Based on international guidelines outlining milk banking protocols, the maximum recommended frozen storage (−20°C) time for HPDHM is 3–6 months.[12,13,16,17] According to the 2018 HMBANA guidelines, frozen HPDHM expires 1 year after the earliest pumping date of milk within the pool.[18] HPDHM is a valuable commodity with a short shelf-life. Affordability was the most frequently cited barrier of nonuse in a 2013 survey of 183 Level 3 neonatal intensive care units (NICUs).[19] Research to evaluate the feasibility of extending HPDHM expiration dates has the potential to reduce cost barriers associated with a short shelf-life and product waste.

The purpose of this article is to review the current evidence for the storage of HPDHM under refrigerated and frozen storage conditions.

Methods

Search process

This review of published literature was conducted through electronic searches of PubMed, Scopus, Science Direct, Google Scholar, ProQuest Central, and WorldCat Discovery. The electronic search included the following keywords and MeSH terms: (i) human milk; (ii) breast milk; (iii) donor milk; (iv) PDHM; (v) milk banks AND storage; (vi) (donor milk OR human milk) AND pasteurization; (vii) (donor milk OR human milk) AND storage; (viii) (donor milk OR human milk) AND refrigerated storage; (ix) (donor milk OR human milk) AND frozen storage; (x) (donor milk OR human milk) AND (storage OR pasteurization); (xi) (donor milk OR human milk) AND (storage OR bank) AND pasteurization; (xii) (human milk OR human milk) AND (storage OR pasteurization) AND (bioactive OR immune OR antimicrobial); and (xiii)

(donor milk OR human milk) AND (storage OR freez* OR refrig* OR processing) AND (pasteuriz*).

Inclusion and exclusion criteria

To be included in this review, a study must have been published between 1985 and May 2018, when the search was conducted. This cutoff date was chosen based on the establishment of HMBANA and its milk processing protocols. Studies were required to be peer reviewed and include the primary outcome of the effects of extended storage, either under frozen (typically −20°C) or refrigerated (typically 4°C) conditions. Only studies examining donor HM that had undergone the Holder method of pasteurization were included, whether explicitly stated or as evidenced by the processing protocol used at the milk bank from which it was acquired. HPDHM fortified with HM fortifiers were also included. Studies assessing colostrum were not included. Studies were also excluded if they did not describe the length of storage time at a given temperature or the method of pasteurization employed, if they did not report outcomes specifically for HPDHM, or if the heat sterilization process differed from the Holder method, such as high temperature–short time, ultra-high temperature, or extended shelf life.

Data extraction

The following information was extracted for each study: author; year; title; type of milk; sample size; fortification status; storage temperature; storage duration; outcomes measured; and findings. Two researchers independently reviewed all studies for inclusion/exclusion criteria and results, and differences were resolved through discussion.

Results

Initially, 19 studies that included HPDHM were identified. Three (16%) did not describe the length of storage time,[20–22] one (5%) did not report outcomes specifically for HPDHM,[23] and one (5%) was not peer reviewed,[24] leaving a final total of 14 studies included in this review (Table 1).

Refrigerated storage of HPDHM

Five studies examined the storage of unfortified HPDHM under refrigerated conditions (Table 2). Storage times ranged from 24 hours to 90 days, and analytes assessed included microbial growth, total protein, bactericidal capacity, lysozyme activity, secretory immunoglobulin A (sIgA) activity, and total ganglioside concentrations. One of these studies included pilot information on fortified HPDHM, although significance was not independently assessed.[28]

Table 1. Summary of Refrigerated and Frozen Storage Studies Using Holder-Pasteurized Donor Human Milk

Author(s)	Year	Storage conditions (°C)	Storage duration	Sample size	Outcome(s) measured
Lepri et al.[25]	1997	−20	90 Days	*n* = 16 single-donor samples	Lipids: modified Folch method, and thin-layer and gas chromatography l-lactate: biosensor Degree of lipolysis: gas chromatography
Silvestre et al.[26]	2008	4–6	72 Hours	*n* = 10 single-donor samples	Bactericidal capacity: *Escherichia coli* viability assay
Vieira et al.[27]	2011	−20	24 Hours	*n* = 57 single-donor samples	Macronutrients: Infrared human milk analyzer (MilkOScan by Foss)
Cohen et al.[28]	2012	4	122 Hours	*n* = 22 previously pooled samples	Bacterial growth: standard plate count method
García-Lara et al.[29]	2013	−20	180 Days	*n* = 34 individual samples from 28 donors	Macronutrients: Infrared Human Milk Analyzer (MIRIS, Sweden)
Vázquez-Román et al.[30]	2014	−20	90 Days	*n* = 36	Fat and energy content: creamatocrit (Lucas method)
Borgo et al.[31]	2015	−18	240 Days	*n* = 1 sample from single donor	Saturated and unsaturated FA: gas chromatography, nuclear magnetic resonance, infrared spectroscopy
Vickers et al.[32]	2015	4	0–96 Hours, 9 Days	*n* = 42 previously pooled samples (2–5 donors per pool)	Bacterial growth: HMBANA Standard Operating Procedure for Culturing PDHM
Marinković et al.[33]	2016	−20	30 Days	*n* = 10 single-donor samples	Antioxidative properties: static oxidation–reduction potential (ORP) measurement; oxygen radical absorbance capacity (ORAC) assay; Reflectoquant ascorbic acid test; electron paramagnetic resonance spin-trapping spectroscopy
Vázquez-Román et al.[34]	2016	−20	3 Months	*n* = 40 previously pooled samples	Dornic acidity: titration
Meng et al.[35]	2016	4	7 Days	*n* = 13 single-donor samples	Aerobic bacteria and coliform count: Petrifilm Total protein: Bicinchoninic acid (BCA) assay Lysozyme activity: *Micrococcus lysodeikticus* based turbidimetric assay IgA activity: kinetic ELISA
Kanaprach et al.[36]	2018	−20	6 Months	*n* = 40 single-donor samples	Intestinal cell growth-promoting activity: fetal intestinal growth assay Antimicrobial effect against *E. coli*: antimicrobial assay
Salcedo et al.[37]	2018	4	90 Days	*n* = 5 single-donor samples	Gangliosides concentrations: ultrahigh-performance liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS)
de Waard et al.[38]	2018	−20	12 Months	*n* = 34 single-donor pools	Bacterial growth: blood and CLED agar Macronutrients: Human Milk Analyzer (MIRIS, Sweden)

HMBANA, Human Milk Banking Association of North America; ELISA, enzyme-linked immunosorbent assay; IgA, immunoglobulin A; PDHM, pasteurized donor human milk; FA, fatty acid; CLED, cysteine- lactose- and electrolyte-deficient.

Table 2. Effects of Extended Refrigerated Storage on Components of Holder-Pasteurized Donor Human Milk

Component	Duration	Findings	Author
Microbial growth	24–122 Hours	No significant change	Cohen et al.[28, a]
	7 Days	No significant change	Meng et al.[35]
	9 Days	No significant change	Vickers et al.[32]
Total protein	7 Days	No significant change	Meng et al.[35]
Bactericidal capacity	72 Hours	No significant change	Silvestre et al.[26]
Lysozyme activity	7 Days	No significant change	Meng et al.[35]
IgA activity	7 Days	No significant change	Meng et al.[35]
Gangliosides			
GM3	90 Days	No significant change	Salcedo et al.[37]
GD3	90 Days	No significant change	Salcedo et al.[37]
Total gangliosides	90 Days	No significant change	Salcedo et al.[37]

[a]Indicates studies that also included fortified Holder-pasteurized donor human milk.

Effects on microbial growth. Currently, there is no consensus definition of "acceptable levels" of bacteria in HM and special considerations must be made for infants in the NICU with compromised immune systems. Regarding healthy, term infants, Meng et al.[35] suggest two options: (i) use the levels set for Pasteurized Milk Ordinance (PMO) for Grade A pasteurized bovine milk (4.30 log colony-forming unit [CFU]/mL), or (ii) set the maximum level as that which is present in the milk in a feeding container immediately after exposure to the microflora in an infant's mouth through bottle or cup feeding (average 2.8 log CFU/mL). In their 2016 study, Meng et al.[35] found that, after 7 days of storage at 4°C, HPDHM consistently had bacterial levels below both the PMO standard and the more stringent constraints set in option 2. The aerobic bacteria count for HPDHM stored at 4°C was 0.0 log CFU/mL at all time points up to 7 days.[35]

These data support similar findings by Cohen et al. who, in a 2012 study, found no bacterial growth in 22 samples of HPDHM that were thawed and refrigerated for 24–122 hours of routine NICU handling.[28] However, 33% (2/6) bottles of fortified HPDHM exhibited bacterial growth. A 2015 study by Vickers et al.[32] found that there was no evidence of microbial growth in HPDHM when thawed and stored at 4°C for up to 9 days. This study utilized 42 randomly selected samples of HPDHM from a HMBANA milk bank. Study milk handling protocol aimed to mimic that which may be found in an NICU feeding room and, on average, the refrigerator was opened 27 times per day.[32] These data suggest that unfortified HPDHM maintains its antimicrobial defenses and remains free of microbial growth when stored at 4°C for up to 9 days.

Effects on macronutrient concentration. One published study has addressed the retention of macronutrients during refrigerated storage of HPDHM, and only protein concentration was assessed. In the 2016 study by Meng et al., HPDHM stored at 4°C exhibited no significant change in total protein concentration ($p = 0.27$) between 0 and 7 days.[35]

Effects on bactericidal capacity and bioactive factors. There are two studies that examine the impact of refrigerated storage on bactericidal capacity and the activity of immunological factors in HPDHM. In their 2008 study, Silvestre et al. determined that the bactericidal capacity of

HPDHM against *Escherichia coli* exhibited no significant changes during 72 hours of refrigerated storage (at 4–6°C).[26] In 2016, Meng et al. reported no significant changes in the activity of lysozyme ($p = 0.77$) and sIgA ($p = 0.49$) after 7 days of refrigerated storage.[35] In 2018, Salcedo et al.[37] published a study looking at the effects of heat treatment and storage time on the concentration of gangliosides in HM. Gangliosides are glycolipids primarily associated with the milk fat globule membrane, and their content and profile constituents vary throughout lactation. GD3 (Neu5Ac α2-8 Neu5Ac α2-3 Gal β1-4Glc β1-1 ceramide) is most abundant during the first few days of lactation, whereas GM3 (Neu5Ac α2-3 Gal β1-4Glc β1-1 ceramide) is found in the highest proportion in mature HM.[39] Salcedo et al. found that storage for up to 90 days at 4°C had no significant impact on either total or specific ganglioside content in HPDHM.[37]

Table 3. Effects of Extended Frozen Storage at −20°C on Components of Holder-Pasteurized Donor Human Milk

Component	Duration	Findings	Author
Bacterial growth	8 Months	No significant growth	de Waard et al.[38]
Total protein	24 Hours	Significant decrease	Vieira et al.[27]
	8 Months	Significant increase	de Waard et al.[38]
Nitrogen	6 Months	No significant change	García-Lara et al.[29]
Total carbohydrate	6 Months	Significant decrease	García-Lara et al.[29]
	8 Months	No significant change	de Waard et al.[38]
Lactose	24 Hours	No significant change	Vieira et al.[27]
l-Lactate	90 Days	Decrease (significance not assessed)	Lepri et al.[25]
Total fat	24 Hours	Significant decrease	Vieira et al.[27]
	3 Months	Significant decrease	Lepri et al.,[25] Vázquez-Román et al.[30]
	6 Months	Significant decrease	García-Lara et al.[29]
	8 Months	No significant change	de Waard et al.[38]
Fatty acids	240 Days	Varied by fatty acid	Borgo et al.[31]
Degree of lipolysis	3 Months	Increase (significance not assessed)	Lepri et al.[25]
Energy	3 Months	Significant decrease	Vázquez-Román et al.[30]
	6 Months	Significant decrease	García-Lara et al.[29]
	8 Months	No significant change	de Waard et al.[38]
Bactericidal capacity	3 Months	No significant change	Kanaprach et al.[36]
	6 Months	Significant decrease	Kanaprach et al.[36]
Antioxidative properties			
Superoxide dismutase	30 Days	No significant change	Marinković et al.[33]
Glutathione peroxidase	30 Days	No significant change	Marinković et al.[33]
Glutathione reductase	30 Days	No significant change	Marinković et al.[33]
Ascorbate concentration	30 Days	No significant change	Marinković et al.[33]
Dornic acidity	3 Months	Non-clinically significant decrease	Vázquez-Román et al.[34]
Intestinal cell growth-promoting activity	6 Months	No significant change	Kanaprach et al.[36]

Frozen storage of HPDHM

While storing raw and pasteurized HM at −80°C minimizes changes to many properties, it is impractical for milk banks and neonatal units primarily due to its expense.[40,41] Freezer storage at −20°C is much more commonplace. This section summarizes the findings from the nine studies

published examining the impact of frozen storage conditions on pH and microbial growth, as well as the retention of macronutrients, immunological activity, and enzymatic activity (Table 3).

Effects on microbial growth. Only one study assessed microbial growth in HPDHM under frozen storage conditions. In a 2018 study, de Waard et al. found that HPDHM stored at −20°C for 12 months remained free of microbial growth for the first 8 months.[38] Microbial analysis at 10 and 12 months revealed positive cultures in HPDHM samples from 17% to 28% of donors; however, study samples were drawn postpasteurization and it was unclear whether this occurred under sterile conditions, which may have influenced results.

Effects on macronutrient concentrations. Six published studies have assessed the impact of frozen storage on macronutrient retention in HPDHM. Five studies examined total fats, three examined total protein or nitrogen, three assessed carbohydrates, and one assessed individual fatty acid profiles.

A 1997 study by Lepri et al.[25] found that, between 0 and 35 days of frozen storage, total fat content of HPDHM decreased only slightly (25.08 mg/mL ±0.54 to 24.67 mg/mL ±0.52), then notably after 70 (23.60 mg/mL ±0.58) and 90 days (23.32 mg/mL ±0.55) of frozen storage. This represented a −7.55% change between baseline and day 90.[25]

Others have also reported a decline in the fat content of HPDHM during extended frozen storage. In a 2011 study by Vieira et al., after 24 hours of storage at −20°C, HPDHM showed significant decreases in mean fat (5.5%, $p < 0.001$) compared with never-frozen HPDHM.[27] In their 2013 study, García-Lara et al. found that there were small but significant decreases in the fat (−0.13 g/dL, 2.8% relative decrease, $p = 0.001$) and energy (−1.55 kcal/dL, or −0.46 kcal/oz, 2.2% relative decrease, $p = 0.001$) content of HPDHM after 180 days of frozen storage.[29] Importantly, authors noted that, while these declines were of low magnitude, when the impact of pasteurization on fat content was taken into account (3.5% relative decrease), the total reduction was 6.2%. This reveals a more clinically relevant issue with regard to the retention of energy for preterm infants who are the primary recipients of HPDHM, given a potentially cumulative detrimental impact associated with multiple processes, including pasteurization and storage.

In their 2014 study, Vázquez-Román et al. found that the fat content of HPDHM decreased by 0.39 g/dL (−15.08% relative change, $p = 0.01$) after 30 days of frozen storage, but there was no significant change at 60 days (0% relative decrease, $p = 0.996$) or 90 days (+6.5% relative change; $p = 0.580$) of frozen storage compared with baseline using creamatocrit as the method for fat assessment.[30] The aliquots prepared for the various storage conditions were homogenized by rocking them in an arc-like fashion 10 times. This might not have been enough to thoroughly mix the study samples, which potentially explains why there were differences in the samples at 30 days, but not at 60 and 90 days compared with baseline. Additionally, while there is a strong correlation between the creamatocrit value and the lipid content of HM,[42] the authors point out the limitations of measuring fat content with this method. There is evidence that the fat globule ruptures during frozen storage and subsequent thawing.[43] This breakdown, along with the continued activity of lipoprotein lipase, causes an increase in free fatty acids. This results in a more tightly packed cream layer and, as this is what is measured in a creamatocrit test, can

produce a false decrease in the creamatocrit reading, which would misrepresent the actual fat (and energy) content of the HM.[44]

Borgo et al., in a 2015 study, assessed the impact of extended freezer storage (−18°C) on the concentrations of specific saturated and unsaturated fatty acids in HPDHM over 8 months, with measurements taken every 30 days.[31] They reported upward, downward, and quadratic trends in several saturated and unsaturated fatty acids. However, there were major limitations to this work, including the fact that it involved a single sample from one donor, that two time points were dropped because of inconsistent findings, and that the study did not describe how the sample was mixed during aliquoting, which may impact whether all subsamples had similar fat content.

In 2018, de Waard et al. reported no significant changes in total fat or energy contents, during 8 months of frozen storage, but a significant increase in protein content (13.4% relative increase, $p = 0.037$).[38] This is in contrast to findings by Vieira et al. in 2011, who reported a significant decrease in protein concentrations (3.9%, $p < 0.001$) after 24 hours of frozen storage.[27] In a 2013 study by García-Lara et al., there was no significant change in total nitrogen, which represents protein and nonprotein nitrogen compounds, in HPDHM during 6 months of frozen storage.[29]

The 2011 study by Vieira et al. reported no significant change in lactose concentrations ($p = 0.427$) after 24 hours of frozen storage.[27] Similarly, de Waard reported stable carbohydrate composition over 8 months of frozen storage.[38] In the 2013 study by García-Lara et al., there was a small but significant decrease in the carbohydrate content (defined as lactose plus oligosaccharides, −0.08 g/dL, 1.7% relative decrease, $p = 0.006$).[29]

Overall, these studies suggest that the total fat in HPDHM decreases by 3–8% between 24 hours and 180 days of frozen storage, including the nonsignificant reduction reported by de Waard et al.[38] These decreases correspond to a significant reduction in energy content. Research reflects stable carbohydrates during extended frozen storage and inconsistent effects on total protein in HPDHM.

Effects on bactericidal capacity and bioactive factors. As part of their 2018 study, Kanaprach et al. assessed the effects of extended storage on the growth-promoting activity of fetal intestinal cells and the antimicrobial defenses against *E. coli* in raw and HPDHM.[36] The authors reported that the antimicrobial activity remained constant in HPDHM for up to 3 months of frozen storage (34.0% ± 13.5, compared with 35.9% ± 14.2 at baseline), but exhibited a significant decline at 6 months (−76.1% ± 23.5, $p < 0.005$; −323.8% change), indicating an increase in bacterial growth. The HPDHM exhibited no significant changes in the growth-promoting activity on fetal intestinal cells.

Effects on antioxidative capacity. There is only one published study assessing the antioxidative capacity of HPDHM during extended storage under any condition. In 2016, Marinković et al.[33] found that Holder pasteurization and storage at −20°C for 30 days did not affect static oxidation–reduction potential or total nonenzymatic antioxidative capacity. While Holder pasteurization caused a significant reduction in ascorbate, superoxide dismutase, and glutathione peroxidase activity in HM, frozen storage did not lead to any further changes.[33]

Effects on pH, acidity, and osmolality. Two studies looked at changes in acidity in HPDHM under frozen storage conditions. In 1997, Lepri et al. found that the concentration of l-lactate remained constant in HPDHM during the first 35 days of frozen storage; however, after 70 and 90 days, there was an 18% decrease (significance not assessed).[25] Authors speculated that this decrease was due to degradation or a change in optical isomeric form. In a 2016 study, Vázquez-Román et al.[34] found that, from baseline to week 1 of storage at −20°C, there was a nonclinically significant decrease in the Dornic acidity (3°D to 2°D, $p < 0.05$) of HPDHM, and this reading remained constant over the course of the 3-month study period. Dornic acidity is an alternative measure of acidity specific to milk. This method determines the total titratable acidity and results are expressed in Dornic degrees, with milk measuring ≥8°D classified as too acidic for pasteurization.[45] The authors hypothesized that the limited change in Dornic acidity of HPDHM, compared with significant changes in raw HM, was due to inactivation of lipase enzymes during pasteurization.

Discussion

Summary of findings

It is impossibly difficult to condense data presented in the existing literature into one tidy statement. There is very little overlap among these studies with regard to the components assessed, analytical methods used, and the length of study period adopted. It would be safe to conclude that extended storage affects some aspects of HPDHM more than others.

Under refrigerated storage conditions, the most studied component of unfortified HPDHM was bacterial growth. All studies reported no bacterial growth in refrigerated unfortified HPDHM, with the longest study duration of 9 days.[28,32,35] Importantly, two of these studies were designed to reflect feeding room practices, where the refrigerator and the HPDHM bottles were opened multiple times a day.[28,32] Not all studies reflected these relevant clinical conditions, which may have biased their results. There have been only single studies on other components in unfortified HPDHM, with no changes reported in total protein, lysozyme activity, sIgA activity, and gangliosides for 7 days or more in refrigerated conditions. While the growing body of evidence suggests that unfortified HPDHM may be safely stored in the refrigerator for longer than 24 hours, there is limited information on the refrigerated storage of fortified HPDHM, with one small study reporting bacterial growth in one-third of samples mixed with a powder fortifier.[28] Refrigerated storage of fortified HPDHM is an important area for future research given the ubiquitous use of HM fortifiers in the NICU setting.[7] Donovan et al. studied fortified HM (raw and Holder pasteurized) over 24 hours of refrigerated storage and reported that different fortifier types had the potential to change human milk properties, including pH and osmolality.[23]

Under frozen storage conditions, the most studied component of HPDHM was fat, with most studies reporting a small but significant decrease over periods of 24 hours to 8 months. The mixing and handling procedure for study samples was often not reported in the current body of research, which may bias findings about fat, given its propensity to separate from the aqueous layer. Additionally, many studies used milliliter volumes of samples, which may bias findings as

it relates to fat, due to the high ratio of container surface area to sample volume compared with what occurs in multiounce bottles of HPDHM. Future studies, especially as they relate to fat, should describe mixing and handling protocols for HPDHM and test clinically relevant volumes. Carbohydrates appear to be stable during extended freezer storage, whereas findings for protein and other components have been inconsistent or only assessed in a single study, suggesting more research is needed.

Limitations and future implications

Several limitations were observed throughout the review process. Very few studies have been conducted that look exclusively at the effects of long-term storage (refrigerated or frozen) on HPDHM. Most address this issue in conjunction with other treatments and outcomes, and sometimes results specific to HPDHM were difficult to assess. Small samples size is another limitation, along with the use of samples from a single donor rather than samples from pooled HPDHM, which would more closely represent HPDHM found in the NICU. Many studies were not designed to reflect clinical practices or typical storage volumes, which may bias results.

While Holder pasteurization is employed by the vast majority of milk banks,[11–14] future research should also focus on the storage of HM processed with techniques other than Holder, given the availability of other HM products in the market. Differences in storage duration before processing should be accounted for in future studies, as the storage period before processing is also likely contributing to changes in milk characteristics. Study periods should extend to 12 months for frozen storage, and 4–7 days for refrigerated storage given the emerging evidence of microbial purity during these time frames. Primary outcomes should include macro- and micronutrient retention, and the activity of bioactive proteins. Larger sample sizes and the use of pooled donor HM would give more power and relevance to study findings. It would fill a gap if future studies were to distinguish between preterm and term HPDHM. With the knowledge that the vast majority of NICUs utilize HM fortifiers,[7] there is a great need to assess the stability of fortified HPDHM beyond 24 hours of refrigerated storage.

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Disclosure Statement

M.T.P. conceived of the review. H.R.S. conducted the literature search. H.R.S. and M.T.P. reviewed all identified studies. H.R.S. was the primary author. Both authors contributed to revisions and agreed to the final article.

M.T.P. serves in an unpaid capacity on the Board of Directors for the Human Milk Banking Association of North America.

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